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ORIGINAL INVESTIGATIONS

PRINCIPAL RESULTS OF THE STUDY OF A LIVING VACCINE AGAINST INFLUENZA

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During a major influenza epidemic in 1936 the first Soviet strain of the virus (Leningrad-36) was isolated from polecats and successfully adapted to albino mice. During the autumn of that year the first tests were made, in conjunction with M. D. Tushinskii's clinic (with the participation of A. I. Drobyshevskaya, A. A. Korovin, O. M. Chalkina and O. I. Shishkina), of the reactive and immunogenic properties of adapted mouse pulmonary influenza virus, introduced directly into the respiratory passages of adult volunteers. It was found that the instillation or atomization of large droplets of an active 5% suspension of this virus in a certain concentration (about ~~10^{-1}–10^{-2}~~ 10^{6}) causes no clinical manifestations but stimulates the accumulation of antibodies in the secretion of the nasal cavity and in the blood. Meanwhile, inhalation of an aerosol of the same influenza virus suspension caused clinical manifestations after 18–24 hr in some vaccinated subjects, reproducing the basic clinical syndrome of influenzal infection, and was accompanied by a more intensive accumulation of antibodies. Only 18 of the 72 human volunteers, with no homologous virus-neutralizing antibodies in their blood, reacted to inhalation of

virus aerosol, whereas persons whose blood contained antibodies did not respond with clinical reactions to administration of a massive dose of laboratory virus.

It was against this background that in 1937 we began trials of living vaccine from the lungs of albino mice in epidemiological observations covering about 20 000 persons to 1940.

The results of these trials of living pulmonary vaccine, given by instillation into the nasal passages or by inhalation, showed a regular lowering of the attack rate of influenza, significantly by one half, in all cases in which vaccination was begun in time and the vaccine used was specific for the influenza arising among the vaccinated subjects. Even a halving of the attack rate during an epidemic of influenza is statistically significant, for this infection involves up to 20-30% of subjects in control groups.

After 1947 the living pulmonary vaccine was replaced by an improved allantoic lyophilized preparation, produced on developing chick embryos. The great advantage of this preparation was that a vaccine could be obtained free from contaminating bacteria and viruses and the range of immunogenic vaccine strains included in the preparation could be extended, which was not possible when mouse lung vaccine was used. However, the need for lyophilization of the allantois vaccine created additional difficulties in the way of issuing a standardized product, for only if the apparatus used for lyophilization is of high quality can the loss of considerable amounts of active virus or variations in its activity be prevented, both among different batches of vaccine and among the ampules of the same batch. We therefore ceased producing living vaccine in powder form, despite the tempting possibility

of issuing a ready-made preparation suitable for use, and utilizing the vast stores of virus in the allantoic membranes.

The various principles of vaccination against influenza have been evaluated experimentally in the laboratories of A. A. Smorodintsev, M. F. Burnett (Australia) and V. M. Zhdanov, who have demonstrated that living vaccine has clear advantages over killed. This was explained not only by the simpler technique of using the living vaccine, but also by the development in this case of a more intensive local immunity of the respiratory passages, preventing the proliferation of virus in the sensitive tissues of the respiratory tract as a result of the intensive production of antibodies in the portals of entry of the infection.

The theoretical grounds for the use of a living vaccine against influenza are derived from recent information on the pathogenesis and immunology of influenza.

The attack rate of influenza in the period of an epidemic differs considerably in persons with different blood antibody levels. Most cases occur in people with a low level of humoral immunity, those with a moderate antibody concentration (1:20–1:40) are attacked much more rarely, and those with a high antibody level (1:80 or higher) are fully protected against the disease. According to the results of the serological examination of 2064 patients in the course of several epidemics of types A, A1, A2 and B influenza, low antibody titers (below 1:10) were present in the first days of the disease in 89% of patients, average titers (1:20–1:40) in 9% and high titers (1:80 or higher) in only 2% (Table 1).

The movement of influenza in the epidemic and interepidemic period bears an obvious relationship to the immunological indices of the population, determined by

the percentage of susceptible individuals and the mean antibody levels (Table 2).

TABLE 1. Quantitative Concentration of Type A, A1, A2 and B Antibodies in the Blood of Influenza Patients Examined in the Acute Period and during Convalescence (from results of the hemagglutination inhibition reaction)

KEY: 1) Period of disease 2) 1st-3rd day 3) 2-3 weeks later 4) No. of patients with antibody titer as ~~under~~ (2064 altogether) 5) low (< 1:10) 6) absolute 7) average (1:20-1:40) 8) high (1:80-1:320)

TABLE 2. Attack Rate of Influenza during the A1 Influenza Epidemic in 1949 Depending on the Antibody Titer at the Onset of the Epidemic

KEY: 1) Antibody titer at onset of epidemic 2) Low (< 1:10) 3) Average (1:20-1:40) 4) High (1:80-1:320) 5) Adult (542) 6) No. of patients 7) Ills. 8) % of each group falling ill 9) Children (504)

It follows that the practicability of vaccination against influenza cannot be denied on the basis that even in persons recovering from influenza immunity either does not develop at all or is only transient and of too short duration to be developed by artificial vaccination. Although the duration of naturally acquired immunity to influenza is shorter than that to many other virus infections, it can nevertheless by measured in terms of the considerable intervals of time elapsing between two successive epidemics of influenza of the same serotype, amounting to about 2 yr for type A influenza and 3-4 yr for type B. This duration of natural immunity against influenza is quite sufficient for carrying out planned immunization in the presence of an effective apparatus for reproducing immunological changes analogous to the natural epidemic.

These general epidemiological arguments in support of the use of living vaccine to not overcome several serious objective difficulties in the way of active immunization against influenza, and not usually observed during vaccination against other virus infections.

The first difficulty results from the biological properties of the influenza virus as an agent stimulating the development of immunity of relatively short duration even in the conditions of natural infection. It is extremely interesting to discover the mechanism of the immunological shortcomings of the influenza virus and to develop experimental methods of modifying vaccine strains. This would enable combinations to be obtained between the viruses of influenza and more active viruses of other species, which could "correct" the imperfect genetic immunogenic complex of the influenza virus.

During recent years we have observed that attenuated strains of adenoviruses and para-influenza viruses are much more effective if introduced by the same method directly into the respiratory passages. They stimulated more intensive immunological changes in vaccinated individuals than living influenza vaccine.

The second serious difficulty is due to the natural variability of the antigenic properties of the influenza virus, as a result of which every 10-15 yr there is a partial or even radical replacement of the antigenic variants of the causative agent of influenza circulating among the population. It is therefore impossible to produce a standard preparation for the specific prophylaxis of influenza with a constant composition for many years, as is the case for living vaccines against smallpox, yellow fever, poliomyelitis, mumps, and measles. For successful immunization against

influenza, the composition of the vaccine strains, especially the most variable strains of group A virus, must be renewed every 5-10 yr, because of the continuous appearance of new antigenic variants which become dominant within a very short time. It can be stated categorically that the problem of large-scale active immunization against influenza by means of living vaccine would have been solved, had it not been for the need to modify this preparation radically every 5-10 yr.

Although these difficulties are important, there is no cause to overestimate their role in practical immunization against influenza. At the present time, immunization with living vaccine from attenuated immunogenic strains of types A2 and B is the only mass measure capable of lowering the attack rate of influenza significantly among protected persons.

Because of lack of standardization of the quality of the preparation at present marketed, the actual indices of the efficacy of vaccination against influenza vary within wide limits, so that strict control of the technological process and improvement in the methods of administration of the vaccine are required.

A factor of particular importance to the improvement of the quality of living influenza vaccine is the choice of highly immunogenic vaccine strains and the increasing of the concentration of active virus in the final preparation. The vaccine strains composing the living influenza vaccine must be selected from antigenic variants dominant during recent years, corresponding at the present time to serotypes A2 and B. These strains must possess the following properties: low reactivity, intensive proliferation in the upper respiratory tract of susceptible persons, insensitivity to human nonspecific inhibitors, and minimal sensitivity to

immune factors already existing among the population.

The efficacy of a living influenza vaccine depends on the following properties: 1) the specificity of the vaccine strains composing the preparation, determined by the correspondence between their antigenic properties and the dominant strains of types A2 and B influenza virus at the contemporary epidemiological period; 2) the activity of proliferation of the vaccine virus on the mucous membranes of the upper respiratory tract of susceptible persons, determined by the presence of the administered virus in the nasal secretion and the pharyngeal mucus, tested 2 and 4 days after primary vaccination; at the same time the virus does not survive in the respiratory tract of persons receiving two doses of living vaccine; 3) the development of specific humoral immunity to the vaccine strains between 2 and 3 weeks after single, and especially after triple vaccination, which we introduced in 1962 into the practice of active influenza immunization. This important sign of postvaccinal immunity is characterized primarily by the percentage of persons losing their susceptibility to influenza, i.e., acquiring specific antibodies as a result of triple immunization.

(see p. 13)

TABLE 3. Immunogenic Activity of Living Type A2 Influenza Vaccine Depending on the Concentration of Active Virus in the Preparation

KEY: 1) Concentration of virus in 1 ml vaccine (in lg 10) 2) No. of persons in group 3) Reactivity of preparation 4) Indices of immunogenic activity 5) No. of persons with fourfold or greater increase in antibodies 6) Mean antibody titer per person 7) relative increase of antibodies

The efficacy of the living vaccine depends not only on the specificity of

the vaccine strains and their immunogenic activity, but also on the concentration of virus in the final preparation. In order to cause marked immunological changes with the aid of innocuous and maximally attenuated influenzal strains, a large enough dose of active virus, which must be equivalent to a minimum of 100 000 embryonic infection units (Table 3), must be introduced into the respiratory tract of susceptible individuals. In order to meet this requirement, a living vaccine must be prepared with a minimal concentration of ~~6.10~~ 10^6 of virus in 1 ml of the product. In this case 0.5 ml of vaccine, administered in a dilution of 1:5, will contain 100 000 infection units of virus.

The close correlation existing between the immunological and epidemiological efficacy of living influenza vaccine implies that the principal problem in active immunization awaiting solution is to reduce as far as possible the number of susceptible persons, characterized by absence of antibodies in serum diluted 1:5-1:10.

Many years of study of vaccination against influenza have shown that this problem cannot be solved by single immunization, even given an optimal preparation and a high concentration of immunogenically potent virus. In most vaccinated persons single immunization may fail because of the appearance at this time of various obstacles to the proliferation of virus (interference by other viruses, abundant secretion of mucus, and so on). After 8-12 days these obstacles disappear, and if a second vaccination is given, the virus begins to proliferate intensively. Triple vaccination at intervals of 8-12 days leads to a sharp fall in the number of susceptible persons, which often falls from 40-50% after primary immunization to 15-25% after triple vaccination (Table 4). An increase in the number of vaccina-

tions with the living preparation is therefore a most important condition for increasing the efficacy of active immunization against influenza.

see p. 23

TABLE 4. Effect of Frequency of Vaccination on the Immunological Efficacy of Active Immunization against Influenza in Vaccinated Persons (initial antibody titer to used strains 1:10)

KEY: 1) Monovalent A2 or B vaccines 2) Divalent A2+B vaccine 3) Frequency of immunization 4) Single Double Triple 5) Percentage of persons showing 4-fold or greater increase in antibody concentration and index of no. of times by which antibodies increased during study of results with strains indicated 6) Index

The triple immunization of the population with living vaccine which we recommend is practicable only by the use of a single polyvalent preparation containing 2 vaccine strains of serotypes A2 and B. Although this preparation, if administered once only, may display an adverse influence from competition between the individual strains (interference) on the reproduction of the various vaccine strains, this defect is completely eradicated by triple immunization with polyvalent vaccine. The less active strains, inhibited after the primary vaccination by their more active competitors, become predominant after the second and third immunizations, because they have not yet created a specific immunity.

The efficacy of living influenza vaccine is also increased significantly if it is introduced into the upper respiratory tract in a finely dispersed state, increasing the area of contact between the virus and the susceptible tissue, which favors proliferation of the virus (Table 5). At our suggestion the producing institutes supply users with portable atomizers, suitable for use with a definite dose of vaccine.

(see p 3)

TABLE 5. Effect of the Mode of Immunization on the Reactive Properties and Immunological Efficacy of Living Type A Influenza Vaccine in Susceptible Persons (adolescents)

KEY: 1) Mode of immunization 2) Instillation into nasal passages 3) Atomization 4) Inhalation of aerosol 5) No. of persons in group 6) No. of persons giving temperature reaction to administration of vaccine 7) No. of persons showing 4-fold or greater increase in antibody concentration 8) Mean index of increase in antibodies 9) No. of persons remaining susceptible

During the past 15 years the Soviet literature has contained reports describing the epidemiological efficacy of living influenza vaccine. Nevertheless, many cases are also reported in which such vaccination was a complete or partial failure. In each case, the facts and conclusions were correct. The reason for the discrepancy was not that the principle of immunization is itself in doubt, but that the present level of production of influenza vaccine does not permit regular supply and use of preparations absolutely uniform with the guaranteed standard as regards quality.

In 1960-61 the Ministry of Health of the USSR conducted large-scale trials of the epidemiological efficacy of the living influenza vaccine issued by various institutes in Moscow and Leningrad. According to results obtained by the Institute of Virology of the USSR Academy of Medical Sciences from the study of more than 1 million vaccinated persons, considerable differences were observed in the reactive and immunogenic properties of these vaccines. Living vaccine from the Moscow Institute of Virus Preparations halved the attack rate among the vaccinated and gave only moderate reactions; vaccine from the Leningrad Institute of Vaccines and Sera, pro-

duced from our own vaccine strains, lowered the attack rate by a factor of 5.2 and gave only slight reactions. These results do not define the limit of efficacy of the living vaccine, for they were obtained after a single administration of two monovalent vaccines, not absolutely standard in quality (as regards concentration of virus).

As a result of the experience gained from this study of living influenza vaccine, new instructions were drawn up to regulate the production and control of this preparation and to ensure that the following requirements regarding the quality of the preparation and the scheme of its replacement are satisfied.

1. - The use of single vaccine strains most closely related to the antigenic variants of types A and B predominating at the particular time and possessing high immunogenic activity yet not producing reactions, for the production of vaccines.

2. The issuing of a preparation with a maximal concentration of active virus ensuring the administration to the vaccinated person of not less than 100 000 infection doses of virus of each serotype. To satisfy this requirement, for the production of vaccine not only the allantoic virus is used, but also the allantoic membranes of infected chick embryos, careful precautions being taken against inactivation of the virus during subsequent drying and prolonged storage.

3. Triple administration of the vaccine in the fourth quarter of each year at intervals of 8-12 days by means of an atomizer. A divalent vaccine (A2+B) is used for immunization, by pooling equal volumes of monovalent A2 and B vaccines at the time of immunization. The living vaccine is supplied by the manufacturing institute with a portable atomizer.

The marketing of a living vaccine with guaranteed specificity, harmlessness and efficacy will provide a sound basis for the successful control of influenza. Vaccination against influenza must become a mass procedure to be carried out on the entire population of the country. This will not only substantially decrease the incidence of the disease, but also gradually eradicate the reservoir of influenza virus.

In the same way mass prophylaxis must be practised against the virus respiratory infections new to medicine, affecting predominantly infants of preschool age, and caused by adenoviruses, para-influenza viruses, ~~Yeatonis~~ Eaton's pneumonia ~~virus~~ agent, syncytial virus and the virus of the common cold. Because of the higher immunizing properties of most of these new viruses by comparison with influenza virus, the preparation of a new associated living vaccine against these viruses causing mass respiratory infections is the next urgent task.

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TABLE 1

1 Период заболевания	4 Число больных с тем или иным титром антител (всего 361 больных)					
	5 низким (1:10)		7 средним (1:20—1:40)		8 высоким (1:80—1:320)	
	6 абс.	%	6 абс.	%	6 абс.	%
2 1—3-й день болезни	1 838	80	185	9	41	2
3 Через 2—3 недели	121	6	475	23	1 465	71

TABLE 2

1 Титр антител в начале исследования	5 Взрослые (542)			9 Дети (504)		
	6 число больных		8 % заболевших в каждой группе	6 число больных		8 % заболевших в каждой группе
	7 абс.	%		7 абс.	%	
2 Низкий (<1:10)	297	57,8	21	460	91,2	32
3 Средний (1:20—1:40)	185	31,1	11	44	8,8	22
4 Высокий (1:80—1:320)	60	11,0	1,5	0	0	0

TABLE 3

1 Содержание вируса в 1 мл вакцины (в lg ID)	2 Число людей в группе	3 Реактогенность препарата	4 Показатели иммунологической активности		
			6 число лиц с увеличением титра антител в 4 раза и более	6 средний титр антител на одного человека	7 кратность нарастания антител
7,4	27	0	21	1:55	6,7
6,4	30	0	16	1:43	4,9
5,4	29	0	10	1:22	4,1
4,4	31	0	5	1:14	2,2
3,4	24	0	2	1:10	1,3

TABLE 4

Preparation Препарат	3 Кратность иммунизации	5 Процент лиц, у которых содержание антител увеличилось в 4 раза и более, и индекс кратности прироста антител при изучении результатов с указанными штаммами			
		A2-96		B-14	
		%	6 индекс	%	6 индекс
1 Моновакцины А2 или В	Однократно } 4	70,0	7,4	52,1	3,8
	Двукратно	82,6	8,1	88,7	7,3
	Троекратно	87,0	9,5	95,7	8,6
2 Дивакцина А2+В	Однократно } 4	57,0	5,0	41,3	3,3
	Двукратно	69,1	7,4	69,0	6,1
	Троекратно	77,5	8,4	79,3	8,7

TABLE 5

1 Способ иммунизации	5 Число людей в группе	6 Число лиц, давших температурную реакцию на введение вакцины	7 Число лиц, у которых содержание антител увеличилось в 4 раза и более	8 Средний показатель прироста антител	9 Число лиц, оставшихся восприимчивыми
2 Закапывание в носовые ходы	98	0	44	2,3	54
3 Пульверизация	71	0	51	3,3	20
4 Ингаляция аэрозоля	90	7	71	4,9	19